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Following screening by these techniques, sequences of interest are typically isolated, optionally sequenced and the sequences used as set forth herein to design new sequences for in silico or other shuffling methods.

Please delete the paragraph beginning at page 96, line 6 and ending at page 96, line 17 and substitute therefor the following new paragraph:

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Generally the charts are schematics of arrangements for components, and of process decision tree structures. It is apparent that many modifications of this particular arrangement for DEGAGGS, e.g., as set forth herein, can be developed and practiced. Certain quality control modules and links, as well as most of the generic artificial neural network learning components are omitted for clarity, but will be apparent to one of skill. The charts are in a continuous arrangement, each connectable head-to tail. Additional material and implementation of individual GO modules, and many arrangements of GOs in working sequences and trees, as used in GAGGS, are available in various software packages. Suitable references describing exemplar existing software are found, e.g., at [aic.nrl.navy.mil/galist/ (on the world wide web) and at cs.purdue.edu/coast/archive/clife/FAQ/www/Q20_2.htm (on the world wide web). It will be apparent that many of the decision steps represented in Figs. 1-4 are performed most easily with the assistance of a computer, using one or more software program to facilitate selection/ decision processes.

Please delete the title above the abstract on page 128 beginning at line 1 and ending at line 2.

In accordance with 37 CFR §1.121 a marked up version of the above-amended paragraph(s) illustrating the changes introduced by the forgoing amendment(s) are provided in Appendix C.

In the Claims:

Please amend the claims by substituting the following claims for the corresponding previously pending claims of the same number(s):

A14 SUB B1

93. (**AMENDED**) A method of producing recombinant nucleic acids or polypeptides, the method comprising:

providing two or more parental nucleic acid or polypeptide sequences;

selecting cross-over sites for recombination between the two or more parental nucleic acid or polypeptide sequences, thereby defining one or more recombinant nucleic acids or recombinant polypeptides that result from a cross-over between at least two of the two or more parental nucleic acids or polypeptides;

determining a recombinant sequence for at least one of the one or more recombinant nucleic acids or polypeptides;

selecting the at least one recombinant sequence in silico for one or more expected activity; and,

synthesizing the at least one recombinant sequence.

94 (AMENDED). The method of claim 93, further comprising providing bridging oligonucleotides which comprise or encode the cross-over sites.

95 (AMENDED). The method of claim 94, wherein synthesizing the at least one recombinant sequence comprises providing fragments of the two or more parental nucleic acids and at least one of [corresponding] the bridge oligonucleotides, hybridizing the fragments and the bridge oligonucleotides and elongating the hybridized fragments with a polymerase or a ligase.

96. The method of claim 93, wherein the two or more parental sequences display low sequence similarity.

97 (AMENDED). The method of claim 93, wherein selecting the at least one recombinant sequence in silico comprises one or more of:

(i) performing an energy minimization analysis of the at least one recombinant sequence;

(ii) performing a stability analysis of the at least one recombinant sequence;

(iii) comparing an energy minimized model of the at least one recombinant sequence to an energy minimized model of one or more of the two or more parental nucleic acids or polypeptides;

(iv) performing protein threading on one or more of the parental or recombinant polypeptides; and,

(v) selecting the cross-over sites for recombination between the two or more parental nucleic acid sequences or polypeptides to occur within regions of structural overlap, thereby determining the sequence of the at least one recombinant nucleic acid or polypeptide;

(vi) performing one or more of: PDA, a branch-and-terminate a combinatorial optimization analysis, a dead end elimination, a genetic or mean-field analysis, or analysis of protein folding by threading, of the at least one recombinant sequence;

(vii) performing PDA of at least one of the two or more parental sequences; or

(viii) comparing a PDA of the at least one recombinant sequence to a PDA of at least one of the two or more parental sequences.

98 (AMENDED). The method of claim 93, wherein the step of selecting cross-over sites for recombination between the two or more parental nucleic acid or polypeptide sequences and the step of selecting the at least one recombinant sequence in silico are performed simultaneously.

99 (AMENDED). A method of producing one or more recombinant nucleic acids or polypeptides, the method comprising:

providing a plurality of first nucleic acid or first polypeptide sequences;

selecting cross-over sequences between the plurality of first nucleic acid or first polypeptide sequences by defining structural, statistical, or logical criteria for the cross-over sequences in silico; and,

artificially synthesizing a plurality of recombinant nucleic acids or polypeptides comprising or encoding the cross-over sequences.

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100. The method of claim 99, wherein the first nucleic acid or polypeptide sequences comprise homologous sequences.

101. The method of claim 99, wherein the first nucleic acid or polypeptide sequences comprise non-homologous sequences.

102. The method of claim 99, wherein the first nucleic acid or polypeptide sequences comprise artificial sequences.

103. The method of claim 99, wherein the first nucleic acid or polypeptide sequences comprise sequences corresponding to naturally occurring nucleic acids or polypeptides.

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104 (AMENDED). The method of claim 99, wherein defining the structural logical or statistical criteria comprises one or more of:

performing structural modeling of at least one of the first polypeptide sequences to define one or more region of structural interest in the at least one first polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt the region of structural interest;

defining a structural or sequence-based motif in at least one of the first polynucleotide or polypeptide sequences to define one or more conserved region in the at least one first polynucleotide or polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt the motif;

identifying one or more nucleotides or amino acids within at least one of the first polynucleotide or polypeptide sequences which shows activity or structural co-variance for one or more desired activities or structural features of the first polynucleotide or polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt the co-variance;

performing an energy minimization analysis of the first polynucleotide or polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt energy minimization of the first polynucleotide or polypeptide sequence;

performing a stability analysis of the first polynucleotide or polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt stability of the of the first polynucleotide or polypeptide sequence at least one recombinant sequence;

comparing an energy minimized model of the first polynucleotide or polypeptide sequence to an energy minimized model of one or more parental nucleic acid from which the first polynucleotide or polypeptide sequence was derived and selecting one or more cross-over sequence to preserve or alter energy minimization of the first polynucleotide or polypeptide sequence;

performing protein threading on one or more first polypeptide sequence and selecting the cross-over sequences to maintain or disrupt protein threading; and,

performing one or more of: PDA, a branch-and-terminate combinatorial optimization analysis, a dead end elimination, a genetic or mean-field analysis, or analysis of protein folding by threading, of the least one of the first polynucleotide or polypeptide sequence.

105 (AMENDED). The method of claim 99, wherein artificially synthesizing a plurality of recombinant nucleic acids comprising or encoding the cross-over sequences comprises synthesizing a plurality of oligonucleotides, one or more of which encodes part or all of one or more of the cross-over sequences and incubating the plurality of oligonucleotides with a polymerase or a ligase, or both a polymerase and a ligase.

Please enter the following new claims

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106. A method of making a recombinant nucleic acid, the method comprising:

providing a plurality of parental character strings encoding a plurality of nucleic acids or polypeptides, which character strings, when aligned for maximum identity, comprise at least one region of heterology;

aligning the character strings;

selecting a plurality of cross-over sites in the character strings;

defining a set of character string subsequences, which set of subsequences comprises at least two subsequences from each of at least two of the plurality of parental character strings;

providing a set of oligonucleotides comprising or encoding the set of character string subsequences, which set of oligonucleotides comprises a plurality of bridging oligonucleotides which comprise or encode the plurality of cross-over sites;

annealing the set of oligonucleotides to each other; and,

elongating one or more members of the set of oligonucleotides with a polymerase, or ligating at least two members of the set of oligonucleotides with a ligase, or both elongating and ligating the set of oligonucleotides with a polymerase and a ligase, thereby producing one or more recombinant nucleic acid.

107. The method of claim 106, further comprising expressing the recombinant nucleic acid to produce one or more recombinant polypeptide.

108. The method of claim 106, wherein the two or more parental sequences display a sequence similarity of less than 50%.

109. The method of claim 106, wherein the two or more parental sequences display a sequence similarity of more than 50%.

110. The method of claim 106, further comprising determining one or more sequence for one or more putative recombinant nucleic acid resulting from in silico recombination of the two or more parental sequences at the cross-over sites, and performing one or more in silico activity simulation for a polypeptide encoded by the putative recombinant nucleic acid.

111. The method of claim 110, further comprising synthesizing the putative recombinant nucleic acid by providing fragments of the parental nucleic acids and at least one of the corresponding bridge oligonucleotides, hybridizing the fragments and the bridge oligonucleotides

and elongating the hybridized fragments and/or bridge oligonucleotides with a polymerase and/or a ligase.

112. The method of claim 106, wherein a parameter which is used in selecting a crossover site includes identification of one or more features of a nucleic acid corresponding to the character string, or of a polypeptide encoded by the nucleic acid corresponding to the character string, wherein the feature is selected from the group consisting of: structural stability, a 3-D energetic constraint, hydrophobicity, co-variation of residues, codon usage, motif distribution, one or more sequence motif, one or more active site, and one or more binding site.

113. The method of claim 106, wherein a parameter which is used in selecting a crossover site includes an analysis of an amino acid composition of one or more polypeptide encoded by a nucleic acid that corresponds to one or more of the parental character strings.

114. The method of claim 113, wherein the analysis includes an analysis of hydrophobicity of the polypeptide, pka of one or more amino acids of the polypeptide, steric bulk of the polypeptide, or entropy of one or more amino acids of the polypeptide.

115. A method of producing recombinant nucleic acids, the method comprising:

providing two or more parental sequences;

selecting a plurality of cross-over sites for recombination between two or more of the parental sequences;

selecting a plurality of bridging oligonucleotides which correspond to the cross-over sites;

predicting at least one recombinant sequence defined by recombination between the parental sequences at the cross-over sites;

selecting the recombinant sequence in silico for one or more expected property of a recombinant nucleic acid that corresponds to the recombinant sequence, or one or more expected property of a polypeptide encoded by the recombinant nucleic acid; and,

synthesizing the recombinant nucleic acid.

116. The method of claim 115, wherein synthesizing the recombinant nucleic acid comprises providing nucleic acid fragments which at least partly correspond to the two or more parental sequences and a plurality of bridge oligonucleotides, hybridizing the fragments and the bridge oligonucleotides and elongating the hybridized fragments with a polymerase or a ligase.

117. The method of claim 115, wherein the parental sequences display sequence similarity of less than 50%.

118. The method of claim 115, wherein selecting the recombinant sequence in silico comprises one or more of:

(i) performing an energy minimization analysis of a polypeptide encoded by a nucleic acid that corresponds to the recombinant sequence;

(ii) performing a stability analysis of a polypeptide encoded by a nucleic acid that corresponds to the recombinant sequence;

(iii) comparing an energy minimized model of a polypeptide encoded by a nucleic acid that corresponds to the recombinant sequence to an energy minimized model of a polypeptide encoded by a nucleic acid that corresponds to one or more of the parental sequences;

(iv) performing protein threading on a polypeptide encoded by a nucleic acid that corresponds to the parental or recombinant sequences;

(v) selecting the cross-over sites for recombination between the two or more parental sequences to occur within regions of structural overlap, thereby determining the sequence of the recombinant sequence;

(vi) performing one or more of: PDA, a branch-and-terminate a combinatorial optimization analysis, a dead end elimination, a genetic or mean-field analysis, or an analysis of polypeptide folding by threading, of the recombinant sequence or a polypeptide encoded by the recombinant sequence;

(vii) performing PDA of at least one of the parental sequences; or

(viii) comparing a PDA of the recombinant sequence to a PDA of at least one of the parental sequences.

119. The method of claim 115, wherein the step of selecting cross-over sites for recombination between the parental sequences and the step of selecting the recombinant sequence in silico are performed simultaneously.

120. The method of claim 115, wherein a parameter which is used in selecting at least one of the crossover sites includes identification of one or more features of one or more of the parental or recombinant sequences, or at least one polypeptide encoded by a nucleic acid corresponding to at least one of the parental or recombinant sequences, which feature is selected from the group consisting of: structural stability, a 3-D energetic constraint, hydrophobicity, co-

variation of residues, codon usage, motif distribution, one or more sequence motif, one or more active site, and one or more binding site

121. The method of claim 115, wherein a parameter which is used in selecting at least one crossover site includes the results of an analysis of an amino acid composition of a polypeptide encoded by a nucleic acid corresponding to the parental or recombinant sequence.

122. The method of claim 121, wherein the analysis includes an analysis of hydrophobicity of the polypeptide, pka of one or more amino acids of the polypeptide, steric bulk of the polypeptide, or entropy of one or more amino acids of the polypeptide.

123. A method of producing cross-over nucleic acids, the method comprising:

providing a plurality of first nucleic acid or first polypeptide sequences;

selecting a plurality of cross-over sequences between the plurality of first nucleic acid or first polypeptide sequences by defining structural, statistical, or logical criteria for the cross-over sequences in silico; and,

artificially synthesizing a plurality of recombinant nucleic acids comprising or encoding the plurality of cross-over sequences.

124. The method of claim 123, wherein the first nucleic acid or polypeptide sequences comprise homologous sequences.

125. The method of claim 123, wherein the first nucleic acid or polypeptide sequences comprise non-homologous sequences.

126. The method of claim 123, wherein the first nucleic acid or polypeptide sequences comprise artificial sequences.

127. The method of claim 123, wherein the first nucleic acid or polypeptide sequences comprise sequences corresponding to naturally occurring nucleic acids or polypeptides.

128. The method of claim 123, wherein defining the structural logical or statistical criteria comprises one or more of:

performing structural modeling of at least one of the first polypeptide sequences to define one or more region of structural interest in the first polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt the region of structural interest;

defining a structural or sequence-based motif in at least one of the first polynucleotide or polypeptide sequences to define one or more conserved region in the first polynucleotide or polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt the motif;

identifying one or more nucleotides or amino acids within at least one of the first polynucleotide or polypeptide sequences which shows activity or structural co-variance for one or more desired activities or structural features of the first polynucleotide or polypeptide sequence, and selecting one or more cross-over sequence to preserve or disrupt the co-variance;

performing an energy minimization analysis of the first polynucleotide or polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt energy minimization of the first polynucleotide or polypeptide sequence;

performing a stability analysis of the first polynucleotide or polypeptide sequence and selecting one or more cross-over sequence to preserve or disrupt stability of the first polynucleotide or polypeptide sequence at least one recombinant sequence;

comparing an energy minimized model of the first polynucleotide or polypeptide sequence to an energy minimized model of one or more parental nucleic acid from which the first polynucleotide or polypeptide sequence was derived and selecting one or more cross-over sequence to preserve or alter energy minimization of the first polynucleotide or polypeptide sequence;

performing protein threading on one or more of the first polypeptide sequences and selecting the cross-over sequences to maintain or disrupt polypeptide threading; and,

performing one or more of: PDA, a branch-and-terminate combinatorial optimization analysis, a dead end elimination, a genetic or mean-field analysis, or analysis of polypeptide folding by threading, of the least one of the first polynucleotide or polypeptide sequence.

129. The method of claim 123, further comprising incubating the plurality of cross-over nucleic acids with a polymerase or a ligase, or both a polymerase and a ligase.

130. The method of claim 123, wherein a parameter which is used in selecting at least one of the crossover sites includes consideration of one or more features of at least one polynucleotide or polypeptide corresponding to at least one of the first polynucleotide or polypeptide sequences, which feature is selected from the group consisting of: structural stability, a 3-D energetic constraint, hydrophobicity, co-variation of residues, codon usage, motif distribution, one or more sequence motif, one or more active site, and one or more binding site.

131. The method of claim 123, wherein a parameter which is used in selecting at least one crossover site includes the results of an analysis of an amino acid composition of a polypeptide corresponding to one or more of the first polypeptide sequences.

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